

## Calcium ions alter monoamine oxidase A activity

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### Introduction

Regulation of the flavoprotein monoamine oxidase A (MAO A) on the mitochondrial outer membrane is now being investigated for a role in cell signalling. In isolated mitochondria (1) and in cell lines (2), MAO A activity was found to increase with increased calcium ion ( $\text{Ca}^{2+}$ ) concentration. *In vivo*,  $\text{Ca}^{2+}$  regulation of MAO A has been reported after manipulation of intracellular calcium using L-type dihydropyridine-sensitive calcium channel agonists (3), and treatment of a monkey with calcium-sodium-EDTA resulted in higher MAO A activity in the brain post-mortem (4). Enhanced MAO A activity has been associated with a rise in oxidative damage (1, 2), and with apoptosis in a neuronal cell line (5). The mechanism of regulation may involve the MAPK pathway but substitution of the potential phosphorylation site did not change the MAO A activity (6). Here, solubilized, purified human MAO A protein and clones of MAO A and B expressed in membranes were used to examine whether MAO activity could be altered by  $\text{Ca}^{2+}$  ions acting directly on the protein.

### Materials and Methods

Human MAO A expressed in *S. cerevisiae* (7) was purified as in previous work (8). Membrane preparations of cloned MAO A and MAO B were obtained from Sigma-Aldrich. Two assays to measure MAO activity were used for all experiments, always in 50 mM HEPES buffer, pH 7.4 at 30°C, with kynuramine as the substrate for MAO A and benzylamine for MAO B. The spectrophotometric assay was conducted in a Shimadzu UV-2101 spectrophotometer as in previous work (8). The fluorescence plate reader assay used peroxidase and Amplex Red to measure the  $\text{H}_2\text{O}_2$  generated (9). All data were analyzed using PRISM v4 (GraphPad Software Inc., San Diego, CA). Kinetic parameters were obtained by non-linear regression using the Michaelis-Menten equation.

### Results and Discussion

*Purified MAO A activity is changed by  $\text{Ca}^{2+}$ .* In the direct spectrophotometric assay where purified MAO A is added last to

initiate catalysis,  $\text{Ca}^{2+}$  does not change the activity. However, if the substrate concentration is low ( $<2 \times K_M$ ) or the MAO A is pre-incubated with 10 nM-100  $\mu\text{M}$   $\text{Ca}^{2+}$  for 10 min before adding kynuramine to initiate the reaction, a increased rate (about 20%) is observed. Above 0.5 mM  $\text{Ca}^{2+}$ , inhibition is seen.

No other ions induced any increase in activity. Sodium (chloride or sulfate), magnesium, manganese and copper ions had no effect on the activity of purified MAO A. Zinc apparently inhibited MAO A strongly with an  $\text{IC}_{50}$  of  $8.1 \pm 0.7$  mM with substrate at  $4 \times K_M$ , but at least some of the apparent inhibition was due to precipitation above 1 mM.

*Purified MAO A activity is increase by EDTA.* Addition of EDTA to the assay medium resulted in an increase in MAO A activity without the need for pre-incubation. The dose-response curve showed that activity increased by 50% at 1  $\mu\text{M}$  EDTA, but increased further between 10 and 100  $\mu\text{M}$  EDTA with a final activity level 200 % of that in HEPES buffer alone. The same results were obtained in buffer made with HPLC grade water for which the ion content was known. Table 1 summarises the activities obtained with EDTA and EGTA. The activity is close to the maximum value with 0.1 mM chelator. Considering the ions present in the HPLC grade water, the ion removed by the chelators could be any of those listed in Table 1. Further experiments are required to determine the binding constant of the unknown ion with EDTA and EGTA.

*Inhibition of purified MAO A by 1 mM  $\text{Ca}^{2+}$ .* After preincubation of MAO A for 10 min with  $\text{Ca}^{2+}$  in HEPES buffer (50 mM, pH 7.4) without chelator,  $\text{Ca}^{2+}$  caused mixed-type inhibition of kynuramine oxidation (data not shown) with a  $K_i$  of 0.3 mM for binding to free enzyme and 0.6 mM for binding to ES ( $\alpha=2$ ). A decline in MAO A activity seen by previous investigators at concentrations of  $\text{Ca}^{2+}$  above 1 mM with fixed concentrations of substrates (1,2) could be due to this inhibition.

*Membrane bound MAO A (but not MAO B) is activated by 1 mM  $\text{Ca}^{2+}$ .* Fig.1 shows that the effect of  $\text{Ca}^{2+}$  on MAO A in its normal membrane environment is a strong activation, as observed in cells and tissues (1,2,3,5).  $V_{\max}$  is doubled by 1 mM  $\text{Ca}^{2+}$ . The small change in  $K_M$  for kynuramine was not significant in the experiment using the coupled Amplex Red assay (Fig. 1), but was doubled from  $43 \pm 4$   $\mu\text{M}$  to  $90 \pm 12$   $\mu\text{M}$  when determined by the spectrophotometric assay. Further work will be done to confirm the parameters.

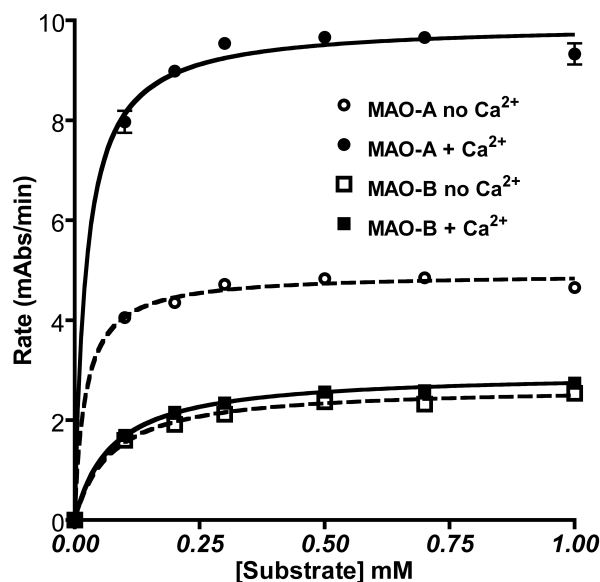
On the other hand, MAO B is clearly not affected by incubation with  $\text{Ca}^{2+}$ , and the kinetic parameters are unchanged (Fig. 1). As previously

reported for synaptic mitochondria (1), the activation of membrane bound MAO A is specific to that isoform.

**Table 1.** Chelators increase the activity of MAO A

Water	Chelator	MAO A activity		
		Mean $\pm$ s.e., mAbsorbance/min		
		Control	0.1 mM	1 mM
HPLC <sup>a</sup>	EDTA	19.2 $\pm$ 0.2	31.8 $\pm$ 0.10	33.6 $\pm$ 0.02
	EGTA		35.5 $\pm$ 0.08	37.0 $\pm$ 0.06
18.2M $\Omega$	EDTA	21.8 $\pm$ 0.1	37.3 $\pm$ 0.05	41.6 $\pm$ 0.04
	EGTA		39.1 $\pm$ 0.07	40.4 $\pm$ 0.19

<sup>a</sup> Contains less than 0.50  $\mu$ M  $\text{Ca}^{2+}$ , 0.41  $\mu$ M  $\text{Mg}^{2+}$ , 0.18  $\mu$ M  $\text{Fe}^{2+}$ , 0.18  $\mu$ M  $\text{Mn}^{2+}$ , 0.17  $\mu$ M  $\text{Ni}^{2+}$ , 0.15  $\mu$ M  $\text{Zn}^{2+}$ .



**Figure 1.** Activation by 1 mM  $\text{Ca}^{2+}$  of membrane bound MAO A but not MAO B. Membrane preparations of cloned human MAO A and MAO B were preincubated for 10 min at 30°C in 50 mM HEPES pH 7.4 without or with 1 mM  $\text{Ca}^{2+}$  before adding 50  $\mu$ L to the reaction mixture (150  $\mu$ L) with kynuramine (MAO A) or benzylamine (MAO B)  $\pm$  1 mM  $\text{Ca}^{2+}$ , and components of the Amplex Red assay.

## Conclusions

In membrane-bound MAO A (but not in membrane-bound MAO B),  $\text{Ca}^{2+}$  enhances activity, doubling the  $V_{\max}$ . In contrast, in purified MAO A, 1 mM  $\text{Ca}^{2+}$  inhibits activity, although activation is observed between 0.01 and 100  $\mu\text{M}$  after 10 min preincubation. Recently, enhancement of activity by membrane attachment was reported (10). Molecular dynamics simulation revealed that membrane attachment facilitated the opening of the substrate tunnel and altered the conformational dynamics (11). The data here are consistent with a conformational change induced by  $\text{Ca}^{2+}$ , the effect of which is different when the enzyme is stabilised in the membrane.

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